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Detection and Analyses of "Difficult to Culture" Microorganisms during Food Processing

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1. Introduction

In these years, roles of so called "difficult to culture" microorganisms in natural environment attract much attention. For example, in soil environment, the percentage of microorganisms capable of forming colonies on solid media is estimated as about 1% of the total living microorganisms. How about in nutritionally rich environment such as food or food wastes?

In recent years, fortunately, we can follow the transition of microflora in natural environment by using modern molecular ecological methods such as DGGE or FISH. In this experiment, we try to confirm the existence of "difficult to culture" microorganisms and clarify the roles of them during food processing and food decomposition by using molecular ecological methods in addition to traditional microbiological methods such as single colony isolation technique.

2. Isolation and characterization of a "difficult to culture" microorganism in the decomposition process of kitchen garbage.

During the analytical process of the microflora during decomposition of kitchen garbage, we observed the existence of so called "difficult to culture" microorganism. We could confirm by DGGE analysis that the major microorganism in the community could not form colonies by normal plating method. Surprisingly, the 16S-rDNA sequence of the DGGE fragment suggests that the existence of "difficult to culture" microorganisms similar to our "difficult to culture" microorganism had been already reported in other habitats such as activated sludge or compost. This microorganism was similar to *Bacillus* species, and named "B Lx"

After enormous efforts, we could finally isolate BLx by the colony formation method, and identified the strain as *Cerasibacillus quisquilarum* gen. nov., sp. nov. after precise taxonomical researches. By quantitative-PCR and FISH methods, BLx was shown to be the major habitat of the degrading process of kitchen garbage, and occupied approximately 30–50% of the total population at the middle stage of the process.

Unexpectedly, BLx showed no distinct ability to degrade food components except gelatinase activity. Moreover, BLx could not utilize major food components such as glucose. On the other hand, BLx showed moderate resisting abilities against high temperature (55°C), high pH (10) and high salt concentration (5%). These resisting abilities of BLx are believed to be one of the reasons that BLx can be the major habitats of the microbial community, because these abilities are considered to be essential to survive during the process we adopted.

3. Analyses of microbial communities during fermentation processes of traditional fermented foods.

We analyzed the transition of microbial communities during Kurozu (black vinegar) making process in Kagoshima Prefecture, because we were highly interested in that saccharification, alcohol fermentation and acetic acid fermentation took place sequentially in one big china jar in this fermentation process.

At present, our achievement on this research is primitive one, and we are just following the experimental results by Prof. Koizumi of Tokyo University of Agriculture, which had been mainly done by traditional isolation method. But, by using modern molecular ecological methods such as DGGE, we could observe the dynamic transition of microflora during the process. The existence of "difficult to culture" microorganisms was also suggested in this process. We hope to clarify the structure and functions of the microbial community during Kurozu making process by using newly developed molecular techniques in addition to traditional microbiological and biochemical methods.